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Human proteins IEF 58 and 57a are associated with the Golgi apparatus

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A mouse monoclonal antibody (mAB 22-II-D8B) raised against lysed transformed human amnion cells (AMA) has been characterized. The mAB decorated the Golgi apparatus in growing and quiescent cultured monolayer cells (fibroblasts and epithelial cells) of various species as determined by double immunofluorescence labeling and colocalization with galactosyltransferase antibodies. It reacted with the acidic human proteins IEF 58 (M_r = 29 000) and 57a, respectively (M_r = 30 000) (HeLa protein catalogue number; [(1982) Clin. Chem. 28, 766]), Golgi staining was also observed in BS-C-1 cells microinjected with mAB 22-II-D8B suggesting that the epitopes recognized by the antibody are most likely located on the cytoplasmic face of the membranes. The precise localization of the antigens to the various cisternae of the Golgi apparatus could not be demonstrated by immunogold cytochemistry on ultrathin cryosections due to either weak reactivity of the antibody or low concentration of the antigens. Immunofluorescence staining with mAB 22-II-D8B of lymphoid human Molt-4 cells and some human tissues failed to reveal any significant staining even though these expressed high levels of both IEF 58 and 57a. These results are taken to imply that the epitopes recognized by mAB 22-II-D8B may be masked in some cell types.

Golgi associated protein; Monoclonal antibody; Cultured cell; Normal tissue; Tumor

1. INTRODUCTION

The Golgi apparatus is the organelle in which proteins destined to the plasma membrane, lysosomes and secretory vesicles are sorted ([1–6] and references therein). The Golgi apparatus is composed of well ordered stacks of membranes in which at least three compartments can be distinguished cytochemically [1–6]. Proteins are believed to move from one compartment to the next by vesicular transport ([3–5] and references therein). Recently, Griffiths and Simons [5] have proposed a model for the Golgi complex in which the last Golgi compartment, termed the *trans* -Golgi network (TGN) is responsible for routing proteins to their final destination.

In this communication we report the characterization of a mouse monoclonal antibody (mAB 22-II-D8B) that stains the Golgi apparatus and reacts with human proteins IEF 58 and 57a (HeLa protein catalogue number; [7,8]). Furthermore, we present evidence suggesting that the epitopes recognized by mAB 22-II-D8B are most likely located on the cytoplasmic face of the membranes.

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Abbreviations: IEF, isoelectric focusing; mAB, monoclonal antibody

2. MATERIALS AND METHODS

2.1. *Cells*

All cultured cells used in this study were grown in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal calf serum and antibiotics (100 units/ml penicillin, 50 μg/ml streptomycin).

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2.2. *Normal human tissues and tumours*

Fresh tumours as well as surrounding normal tissues were obtained from the Pathology Department, Aarhus University.

2.3. *Immunization and monoclonal antibody production*

Human AMA cells lysed in hypotonic saline were used for immunization. Two-month-old BALB/c mice were injected intraperitoneally with the equivalent of about 10^6 cells and the procedure repeated twice at 2-week intervals. After a rest period of 1 week, spleen cells from immunized mice were fused with X63 mycloma cells according to standard procedures [9]. Hybridomas were screened by indirect immunofluorescence microscopy using methanol fixed AMA cells. A hybridoma (mAB 22-II-D8B) that reacted specifically with the Golgi apparatus of AMA cells was cloned twice by limited dilution using feeder cells (macrophages) to stimulate growth.

The procedures for indirect immunofluorescence [10], [^{35}S]methionine-labeling [11,12], two-dimensional gel electrophoresis [13,14], immunoblotting [15,16], microinjection [17,18] and immunogold cytochemistry on ultrathin cryosections [19] have been previously described.

3. RESULTS

3.1. *mAB 22-II-D8B reacts with the Golgi apparatus in cultured cells*

Indirect immunofluorescence staining of AMA cells permeabilized and fixed in methanol (−20°C, 5 min) showed that mAB 22-II-D8B stained the Golgi apparatus (fig.1). Double immunofluorescence staining of AMA cells reacted with mAB 22-II-D8B (fig.1B) and a rabbit polyclonal antibody against human milk galactosyltransferase (found in the lumen of the *trans* Golgi cisternae, fig.1C) [20] showed complete colocalization of the staining. The perinuclear staining was reticular in interphase cells, but looked scattered and fragmented in mitotic cells. The precise localization of the antigens to the various cisternae could not be demonstrated by immunogold cytochemistry on ultrathin cryosections. Thus, specific labeling above the background level was not obtained.

So far, Golgi staining with mAB 22-II-D8B has been observed in cultured monolayer cells (quiescent as well as growing fibroblasts or epithelial cells) of all species analyzed. These include: bat (bat, CCL 88), dog (dog thymus), goat (goat synovial), hamster (CHO), mink (lung, CCL64), monkey (BS-C-1), mouse (3T3), potoroo (PTK1), rabbit (cornea, CCL 60) and rat (SV40 REF 52) (results not shown).

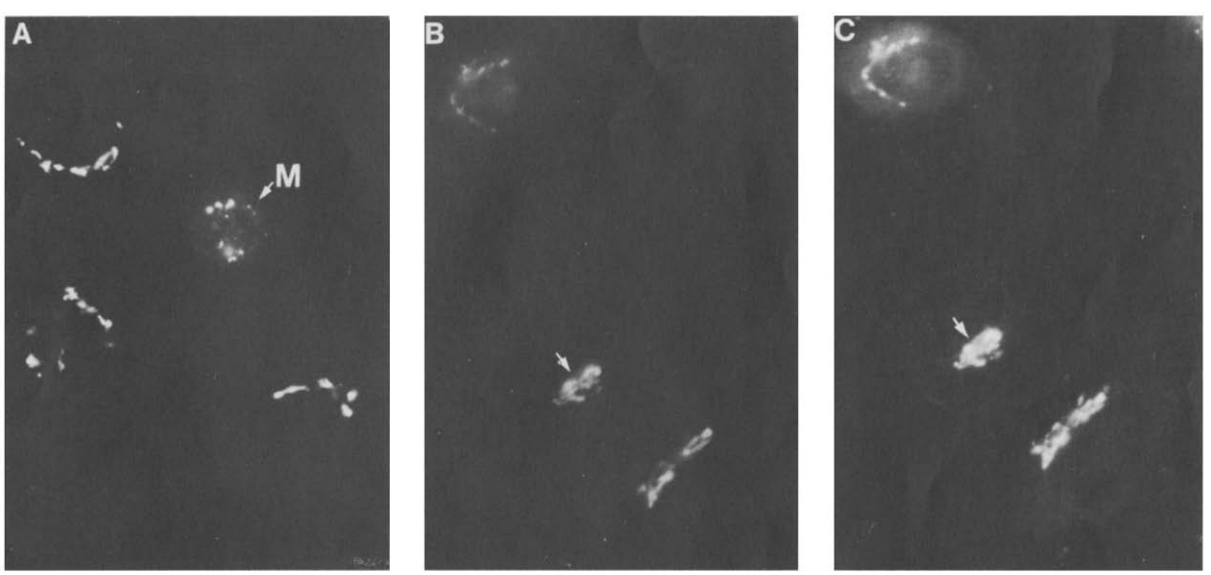

Fig.1. Indirect immunofluorescence of methanol fixed AMA cells reacted with mAB 22-II-D8B. (A) Cells reacted with mAB 22-II-D8B. (B,C) Double immunofluorescence of cells reacted with mAB 22-II-D8B (FITC) (B) and galactosyltransferase antibodies (rhodamine) (C). The arrows in B and C indicate identical cells. M = mitotic cell.

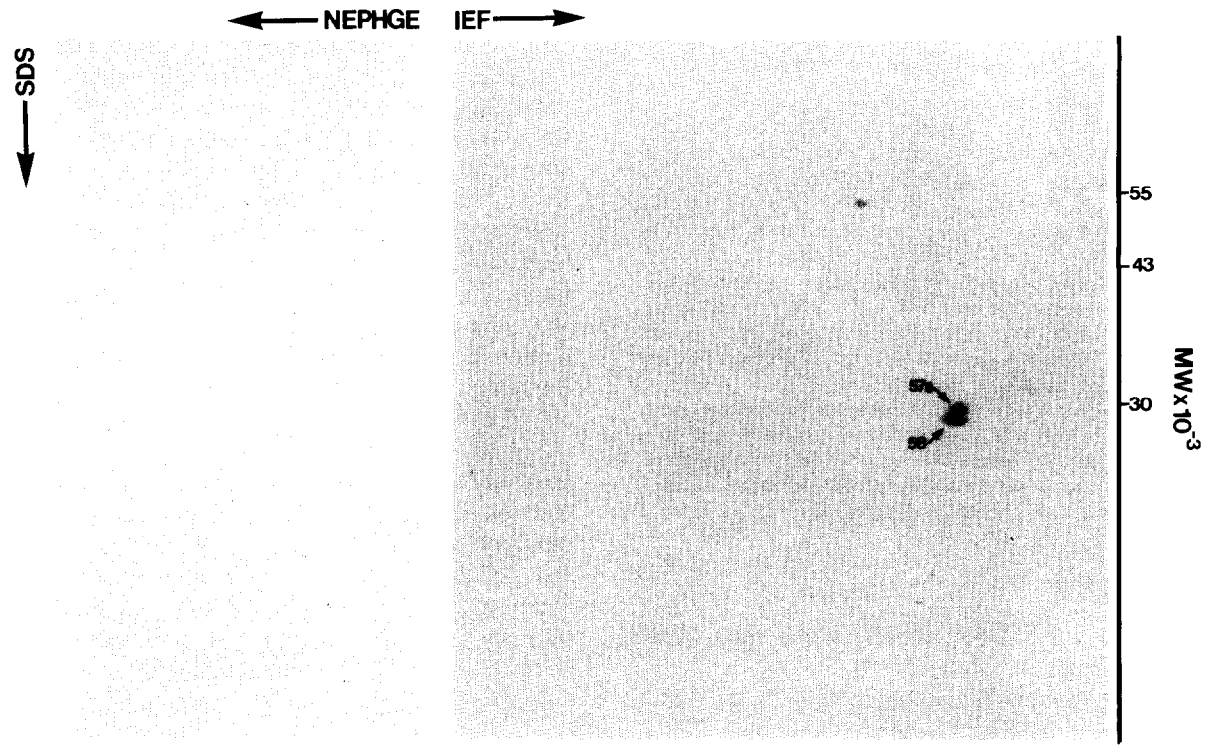

Fig.2. Identification of the antigens reacting with mAB 22-II-D8B by two-dimensional gel electrophoresis and immunoblotting. [^{35}S]Methionine-labeled HeLa cell proteins were separated by two-dimensional gel electrophoresis (NEPHGE, IEF) and transferred to nitrocellulose sheets as described [15]. Antigens were identified by superposition of immunoblots with their corresponding (identical) autoradiograms.

3.2. *mAB 22-II-D8B reacts with the acidic human proteins IEF 58 and 57a (HeLa protein catalogue number)*

The specificity of mAB 22-II-D8B was determined by two-dimensional gel immunoblotting (NEPHGE, IEF) of human AMA cell proteins. As shown in fig.2, the antibody reacts mainly with the acidic proteins IEF 58 (M_r = 29 000, HeLa protein catalogue number; [7,8]) and 57a (M_r = 30 000, HeLa protein catalogue number; [7,8]). For reference, the positions of IEF 58 and 57a are indicated in an IEF gel of [^{35}S]methionine-labeled AMA cell proteins (fig.3). Proteins of similar molecular masses and p*I* have been observed in bat (bat CCL 88), goat (goat synovial), hamster (CHO), mouse (3T3), monkey (BS-C-1) and rat (SV40 REF 52) (results not shown) cultured monolayered cells, although the ratio between these proteins seems to be different.

At present it is not known whether IEF 58 and 57a are related by modification, belong to a family of related proteins, or whether they are unrelated polypeptides sharing common antigenic determinants. Both proteins are synthesized in vitro in a reticulocyte cell free system incubated with polyA$^+$ mRNA obtained from human MRC-5 fibroblasts and their ratio is similar to that observed in vivo (results not shown).

3.3. *IEF 58 and 57a epitopes are most likely located on the cytoplasmic face of the Golgi membranes*

Evidence suggesting that either IEF 58, 57a or both epitopes are localized on the cytoplasmic face of the Golgi membranes was obtained by microinjecting mAB 22-II-D8B into the cytoplasm of BS-C-1 cells. Microinjected cells were fixed in methanol at various times after injection and were reacted with rhodamine-conjugated rabbit antimouse immunoglobulins. Golgi staining was

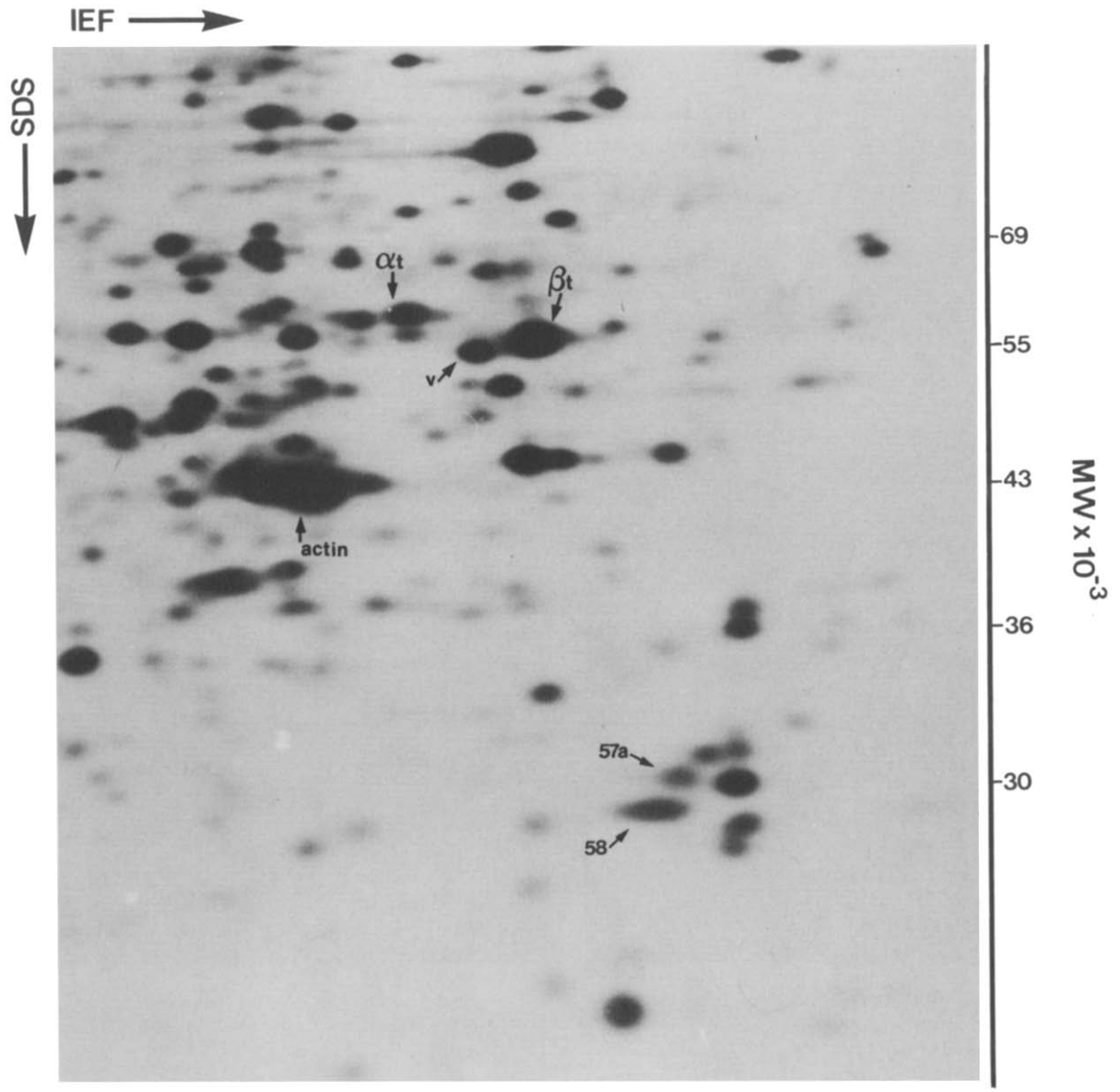

Fig.3. Position of IEF 58 and 57a in a two-dimensional gel map (IEF) of [^{35}S]methionine-labeled proteins from HeLa cells. The position of actin, alpha tubulin (αt), beta tubulin (βt) and vimentin (v) are indicated for reference.

observed early after injection (fig.4A; 20 min after injection) and remained unchanged even after prolonged periods (up to 24 h). No Golgi staining was observed when BS-C-1 cells were injected with antibodies against galactosyltransferase, an enzyme found in the lumen of the *trans* Golgi cisternae (fig.4B) [20]. Taken together these results suggested that mAB 22-II-D8B antigen is accessible to the antibody in intact cells and may be associated with the cytoplasmic face of the Golgi membranes.

3.4. *Epitopes recognized by mAB 22-II-D8B may be masked in some cultured cells and tissues*

Indirect immunofluorescence staining of

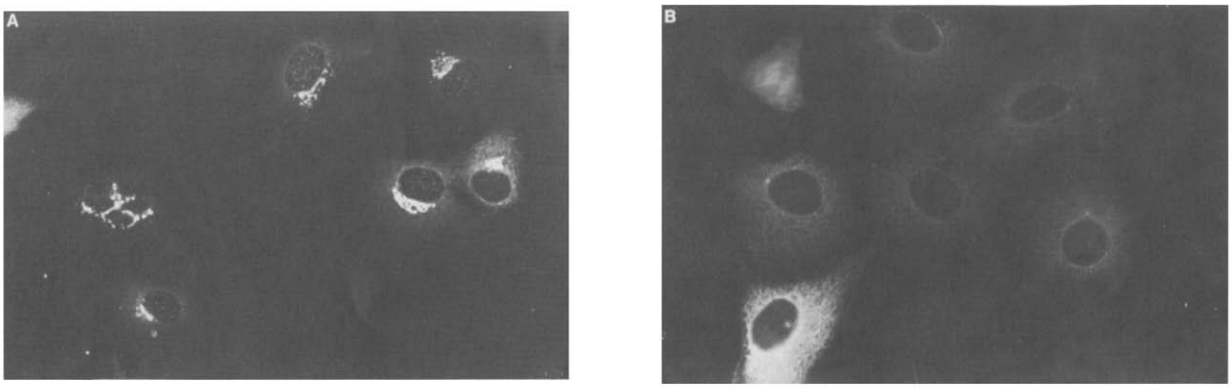

Fig.4. Cytoplasmic microinjection of BS-C-1 cells with (A) mAB 22-II-D8B and (B) galactosyltransferase antibodies. Injected B-SC-1 cells were fixed with methanol 20 min after injection. To visualize the first antibody the fixed cells were reacted with rhodamine conjugated second antibodies. The cytoplasmic staining observed in (B) is usually observed in cells injected with non-immune antibodies.

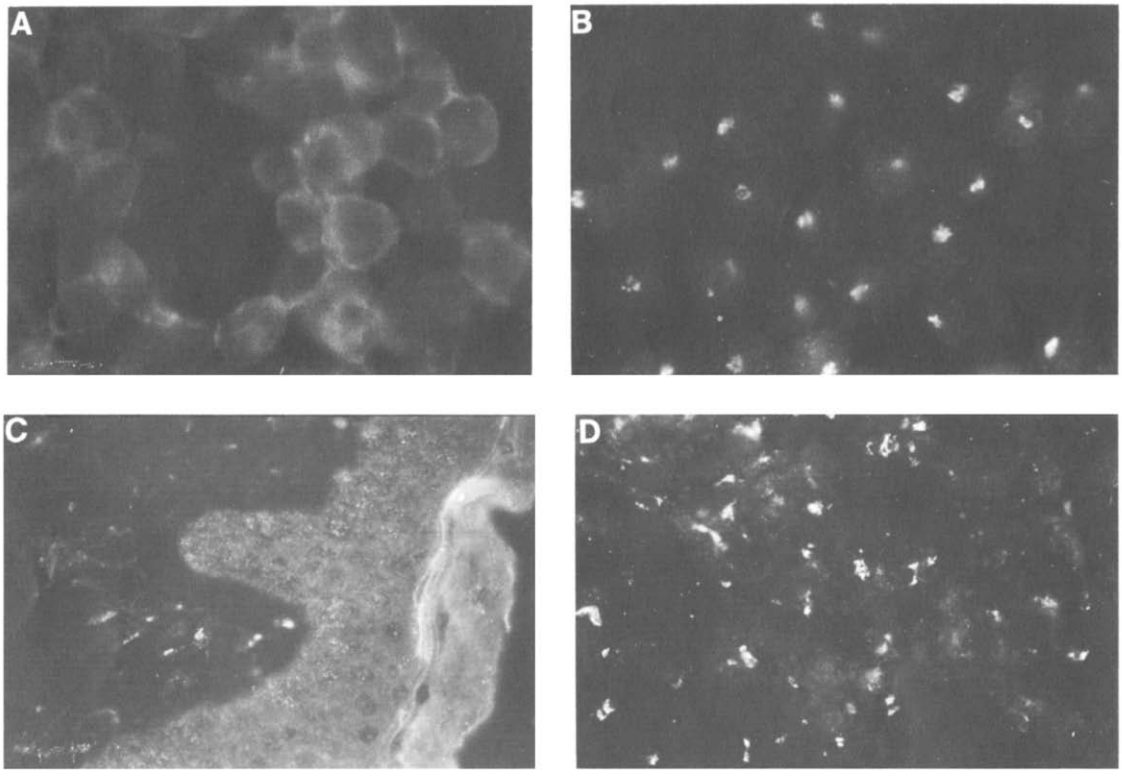

Fig.5. Indirect immunofluorescence of cultured cells and human tissues. (A) Methanol fixed Molt-4 cells reacted with mAB 22-II-D8B. (B) As (A) but reacted with galactosyltransferase antibodies. (C) Methanol-fixed human epidermis reacted with mAB 22-II-D8B and (D) methanol-fixed human basal cell carcinoma reacted with mAB 22-II-D8B.

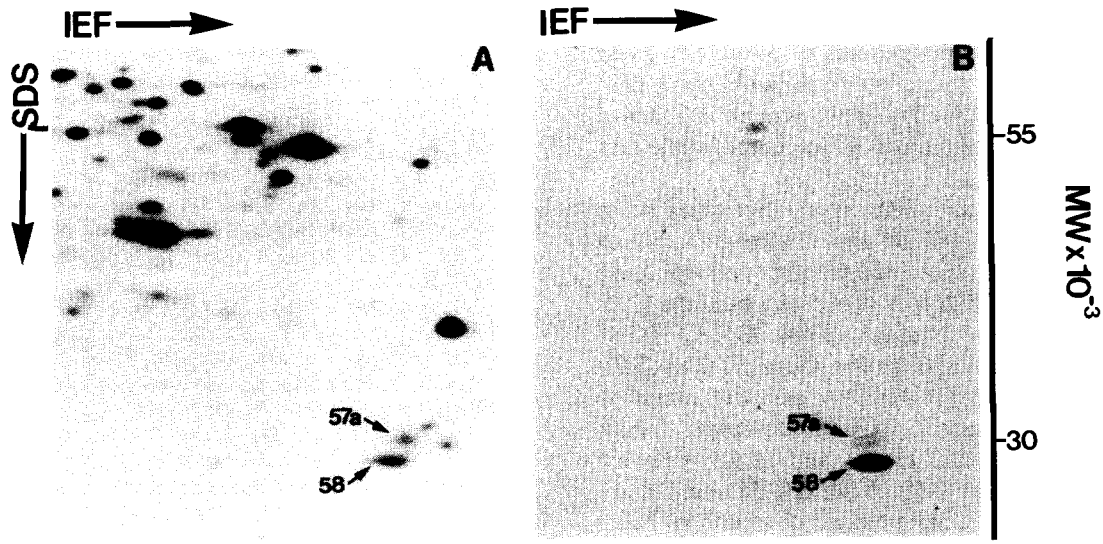

Fig.6. Presence of IEF 58 and 57a in Molt-4 cells. (A) IEF gel of [^{35}S]methionine-labeled proteins. (B) Two-dimensional gel blot of Molt-4 proteins reacted with mAB 22-II-D8B.

methanol fixed human lymphoid Molt-4 cells with mAB 22-II-D8B first suggested that the epitopes recognized by the antibody may be masked in some cell types. These cells do not stain with the antibody (fig.5A) in spite of the fact that they synthesize sizeable amounts of IEF 58 and 57a (fig.6A). Moreover, these proteins react with the antibody in two-dimensional gel immunoblots (fig.6B). Lack of staining with mAB 22-II-D8B is not due to a permeation artifact as these cells stain strongly with galactosyltransferase antibodies (fig.5B). Similar results to those described above have been observed with mouse myeloma cells (X63) and normal human epidermis (fig.5C; note that dermal fibroblasts stain brightly with the antibody). Interestingly, epidermal basal cells in basal cell carcinomas gave a positive staining with the antibody (fig.5D). The latter cells exhibit similar levels of IEF 58 and 57a as total epidermis [21]. Lack of staining with the antibody has been observed in various normal human tissues (brain, heart muscle, lung, skeletal muscle and pancreas; not shown) suggesting that this may be a generalized phenomenon. Some tumours (melanoma, cardiac tumour, lung adenocarcinoma, rectum carcinoma) on the other hand show a bright staining with the antibody similar to that observed in cultured monolayer cells (results not shown).

4. DISCUSSION

This article describes the characterization of a mouse mAB that stains the Golgi apparatus and reacts with human proteins IEF 58 and 57a (HeLa protein catalogue number; [7,8]). By immunofluorescence labeling we found that the monoclonal antibody colocalized with the galactosyl-transferase antibodies [20]. However, we were unable to localize the epitopes to a given cisterna using immunogold cytochemistry on ultrathin cryosections. This may be due to weak reactivity of the antibody, to the effect of aldehyde fixation, and/or to a small amount of the antigens. In the latter case, it should be noted that the labeling efficiency of the gold technique is low [19]. Our observations of a fragmented immunofluorescence labeling of mitotic cells with the monoclonal antibody is in agreement with the finding of Lucocq and Warren [22] of Golgi fragmentation during mitosis.

Evidence based on microinjection of mAB 22-II-D8B, suggested that the epitopes recognized by the antibody may be located on the cytoplasmic face of the Golgi membranes. Whether these proteins are integral membrane proteins or they are just bound to the cytoplasmic domain of the Golgi apparatus is at present unknown. So far, only a few proteins have been localized at the cytoplasmic do-

main of the Golgi apparatus. These include two peripheral membrane proteins of 54 and 86 kDa, respectively [23], and a microtubule-binding protein of 110 kDa [24]. The latter protein is believed to link the Golgi apparatus to the microtubule network [24].

As far as we can judge from molecular mass estimates proteins IEF 58 and 57a are different to nicotinamide adenine dinucleotide phosphatase (medial Golgi) [25,26], *N*-acetyl glucosamine [27], thiamine pyrophosphatase (*trans* Golgi, TGN) [28], sialyl-transferase (*trans* Golgi, TGN) [29], acid phosphatase (TGN) [30] and the mannose 6-phosphate receptor (Golgi complex, small vesicles (clathrin-like coats) and larger vesicles) (for reviews see [31–33]). Also, IEF 58 and 57a are different to other Golgi proteins of unknown function [34,35].

At present, it is not known why the epitopes recognized by mAB 22-II-D8B are masked in some cultured cells and tissues. It is possible that this reflects dissimilarities in the orientation of the molecules in the membrane, resulting in a differential availability of the epitopes. Further studies will be necessary to unravel the molecular mechanisms responsible for the differential reactivity of various cultured cell types and tissues with the antibody.

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